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Original scientific paper

Electrochemical sensor for acetylcholine detection based on WO_3 nanorods-modified glassy carbon electrode

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Abstract

Acetylcholine (ACH) is one of the excitatory neurotransmitter in the human body. It is the most abundant neurotransmitter responsible for triggering the activation of postsynaptic neurons, leading to an excitatory response. ACH plays a crucial role in various physiological processes, including muscle contraction, autonomic nervous system regulation, and cognitive functions such as learning and memory. In this study, an electrochemical sensor was prepared based on WO_3 nanorods modified glassy carbon electrode for the detection of ACH. The WO_3 nanorods provided excellent properties for the electrochemical determination of ACH. The proposed sensor exhibited a wide linear detection range of (0.1 to 400.0 µM) and a low detection limit of 0.025 µM for ACH. These results demonstrate the sensor's high sensitivity in detecting this important neurotransmitter. In addition the developed sensor showed good ability for ACH determination in real samples. This study offers an innovative strategy for the electrochemical detection of ACH, showcasing the potential of nanomaterials in the development of advanced sensing technologies.

Keywords

Cyclic voltammetry; differential pulse voltammetry, modified electrodes, real sample analysis, neurotransmitter

Introduction

Acetylcholine (ACH) is a prominent neurotransmitter found throughout the central and peripheral nervous systems. ACH is vital for various cognitive processes in the human CNS, including memory, learning, attention, sleep, and consciousness. Dysfunction of the cholinergic system has

been clinically linked to certain neuropsychiatric disorders, like Alzheimer's, and Parkinson's diseases. However, the precise effects of ACH in the brain on cognitive functions and neuropsychiatric disorders remain not fully understood. As a result, there is a pressing need to develop rapid and sensitive *in vivo* techniques for measuring ACH [1-3]. To this end, researchers have developed a variety of analytical techniques that enable the reliable and quantitative determination of ACH concentrations in various biological samples, such as LC-MS/MS, fluorescence spectroscopy, high-performance liquid chromatography (HPLC) and colorimetry [4-7]. However, these measurement methods are frequently time-consuming, costly, and necessitate extensive sample preparation before analysis. Electrochemical techniques offer significant advantages for the real-time quantitative analysis of analytes, as they are generally faster, more user-friendly, and can provide continuous and reversible responses without interference from the sample [8-15]. Electrochemical sensors typically consist of a transducer coated with a chemical or biological recognition layer, where the interaction between the target analyte and the sensitive layer provides the analytical information [16-25].

In recent years, the rapid progress of nanoscience has resulted in the emergence of nano-materials with distinctive photonic, catalytic, electronic, and magnetic properties owing to their high surface area, specific morphologies, and various applications [26-35]. Especially, the high surface-to-volume ratio, high chemical stability, prominent electrical conductivity, and biocompatibility of nanostructures can significantly increase their use in electrochemical sensors and biosensors [36-46]. Among various nanomaterials, nanostructured tungsten oxides (WO_3) have attracted considerable attention due to their large surface area and other good properties. WO_3 nanostructures have been widely applied in numerous fields, such as gas sensing, photocatalysis, *etc.* [47-50].

In this study, we developed a sensitive and convenient non-enzymatic electrochemical sensor for ACH detection. The non-enzymatic electrochemical sensors are very advantageous due to their low cost, stability, and easy preparation. The sensor was made by attaching WO_3 nanorods (NRs) to the surface of the glassy carbon electrode (GCE). The electrocatalytic properties of the sensor WO_3-NRs-modified GCE were investigated using cyclic voltammetry (CV), chronoamperometry and differential pulse voltammetry (DPV). WO_3 NR/GCE sensor provides a new method for sensitive detection of ACH.

Experimental

Instruments and chemicals

The electrochemical measurements were conducted using an Autolab PGSTAT 302N system (Eco Chemie, the Netherlands). A Metrohm pH meter (model 710) was used to measure the pH of the solutions. The chemicals, ACH, NaOH, H_3PO_4 and other chemicals used in the study were of analytical grade and were used as received, without any additional purification. These chemicals were purchased from Merck and Sigma-Aldrich companies.

The synthesis and characterization of WO_3 NRs were described in our previous work [51]. Figure 1 shows the FE-SEM image of WO_3 NRs.

Modification of GCE modified with WO_3 nanorods

The bare GCE was carefully pretreated by polishing with an alumina slurry on a polishing cloth. After polishing, the electrode was washed with double-distilled water. Next, 1.0 mg of WO_3 NR was added to 1 mL of double-distilled water. The suspension was then sonicated for approximately 30 minutes to ensure homogeneous dispersion of the nanorods. Lastly, the solvent was allowed to evaporate at room temperature, immobilizing the WO_3 NRs immobilized on the GCE surface. This

immobilization of the synthesized nanomaterial onto the GCE surface was a crucial step in the fabrication of the electrochemical sensor device.

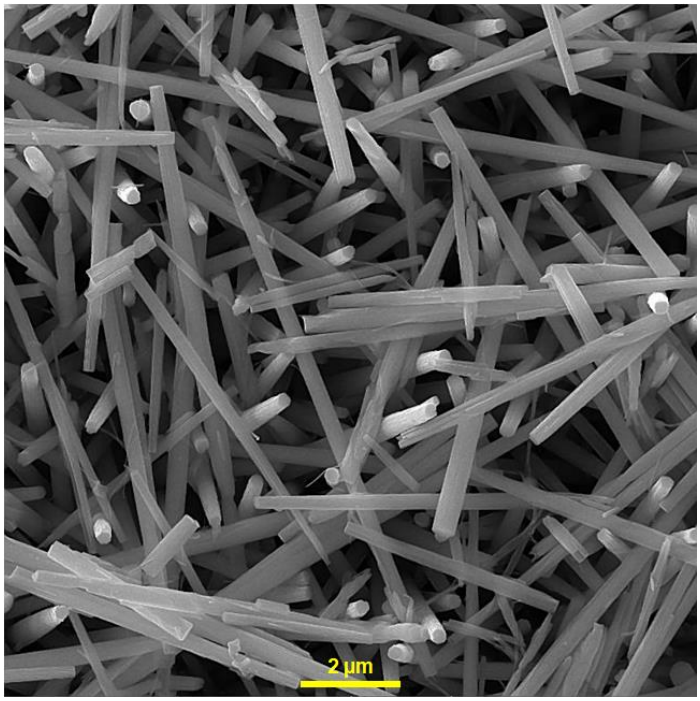

Figure 1. *FE-SEM image of WO_3 NRs*

Results and discussion

Electrochemical activity of ACH on WO_3 NRs/GCE

The electrochemical characteristics of the WO_3 NRc/GCE were investigated using cyclic voltammetry (CV). Figure 2 presents a comparison of the CVs obtained using (a) a bare GCE, and (b) the WO_3 NRc/GCE in phosphate buffer solution (PBS, 0.1M) at pH 7.0 and a scan rate of 50 mV/s.

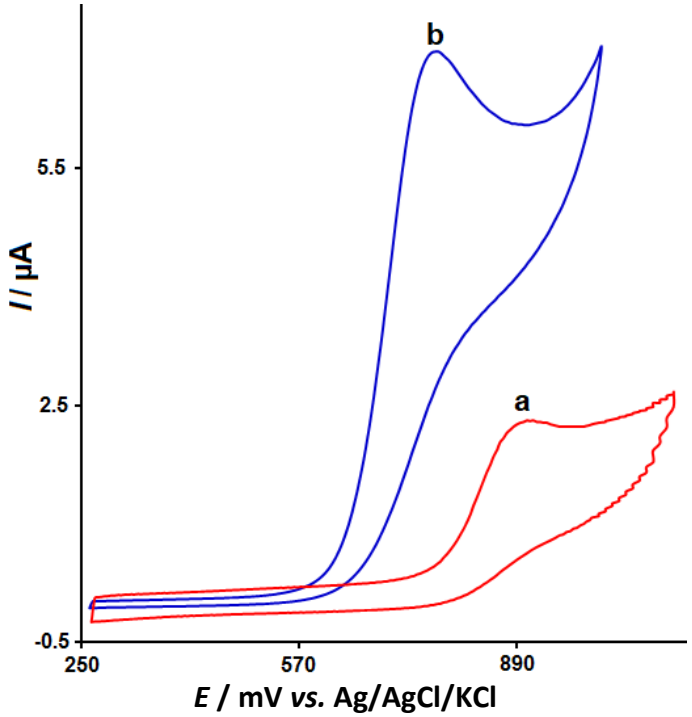

Figure 2. *The CVs of (a) bare GCE and (b) WO_3 NR/GCE in the presence of 100.0 µM ACH in PBS (0.1 M, pH 7.0) and a scan rate of 50 mV s^{-1}*

The CV of the bare GCE showed a weak oxidation peak for ACH in the studied potential range. Compared to the bare (unmodified) GCE, the WO_3 NRs/GCE exhibited significantly higher current responses and lower peak potential towards the oxidation of ACH. This enhanced electrochemical performance can be attributed to the improved specific surface area provided by the WO_3 NRs. The integration of the WO_3 NRs onto the electrode surface increased the effective surface area available for electrochemical reactions. This higher surface area allowed for more efficient electron transfer kinetics and redox processes compared to the bare GCE. These results indicate that modifying the electrode surface with WO_3 NRs/GCE can enhance the sensitivity and reduce the overpotential.

Figure 3 illustrates the impact of varying scan rates on the oxidation currents of ACH. Increasing the scan rate led to an enhancement of the peak currents. Moreover, the linear relationship between the peak currents (I_p) and the square root of the scan rate ($v^{1/2}$) suggests that the oxidation process is diffusion-controlled (Figure 3 inset).

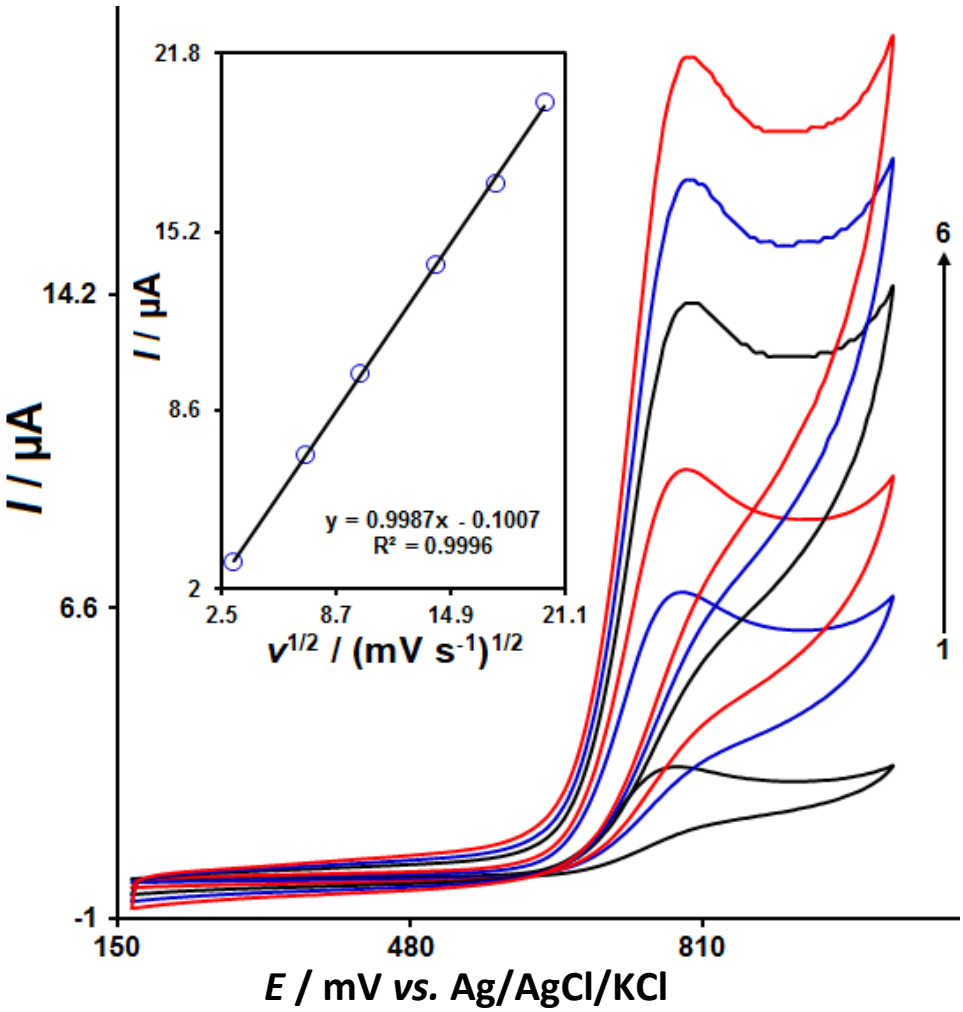

Figure 3. *The CVs of a WO_3 NRs/GCE in PBS (0.1M, pH 7.0) containing 100.0 µM ACH at various scan rates (10 (1), 50 (2), 100 (3), 200 (4), 300 (5), and 400 (6) mV s^{-1}). Inset: the variation of the anodic current as a function of the $v^{1/2}$*

Chronoamperometry studies

After the initial analysis of the CV data, a more in-depth investigation was conducted using the chronoamperometry (CA) method to determine the diffusion coefficient of ACH at the WO_3 NRs-modified electrode. Figure 4 presents a series of chronoamperograms obtained for ACH over the concentration range of 0.1 to 2.0 mM. These measurements were performed at a constant potential of 820 mV versus the Ag/AgCl reference electrode, which corresponds to the anodic oxidation of

ACH. Experimental plots of current versus the square root of time ($t^{-1/2}$) were generated, and the best-fit lines were obtained for different concentrations of ACH (Figure 4, A). The slopes of these linear plots were then plotted against the ACH concentration (Figure 4, B). Using the Cottrell equation, the mean value of the diffusion coefficient (D) was calculated from the slope of the plot in Figure 4B. The determined value of the diffusion coefficient was 3.1×10^{-5} cm^2 s^{-1}.

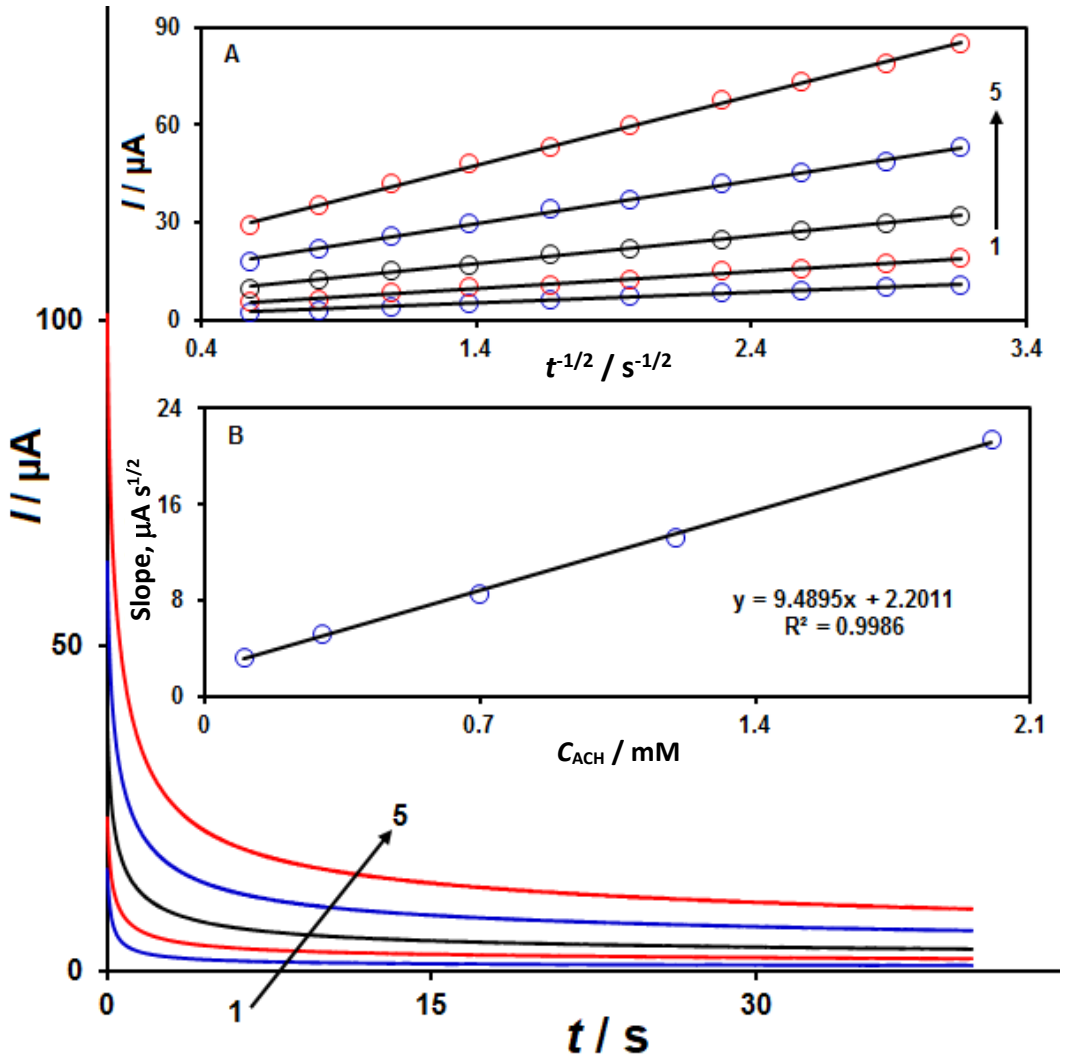

Figure 4. *The chronoamperograms obtained at the WO_3 NRs/GCE in PBS (0.1 M, pH 7.0) for different concentrations of ACH ((1) 0.1, (2) 0.3, (3) 0.7, (4) 1.2, and (5) 2.0 mM). Insets: (A) the plots of current versus $t^{-1/2}$ obtained from the chronoamperograms and (B) the plot of slopes of the straight lines shown in (A) against the ACH concentration*

Calibration curves

Figure 5 presents the differential pulse voltammetry (DPV) curves of the WO_3 NRs/GCE with varying concentrations of ACH. As shown in Figure 5, the current of the DPV responses increases with the increasing concentration of ACH. The relationship between the current and the concentrations of ACH (0.1-400.0 µM) is exhibited in the inset of Figure 5. This linear relationship demonstrates the excellent sensitivity of the WO_3 NRs/GCE sensor towards ACH. Furthermore, the LOD of the WO_3 NRs-modified GCE for ACH was determined to be as low as 0.025 µM. These results indicate that the WO_3 NRs/GCE exhibits exceptional analytical performance, with a wide linear range and a remarkably low detection limit for the electrochemical determination of ACH. This highlights the potential of the developed sensor for practical applications.

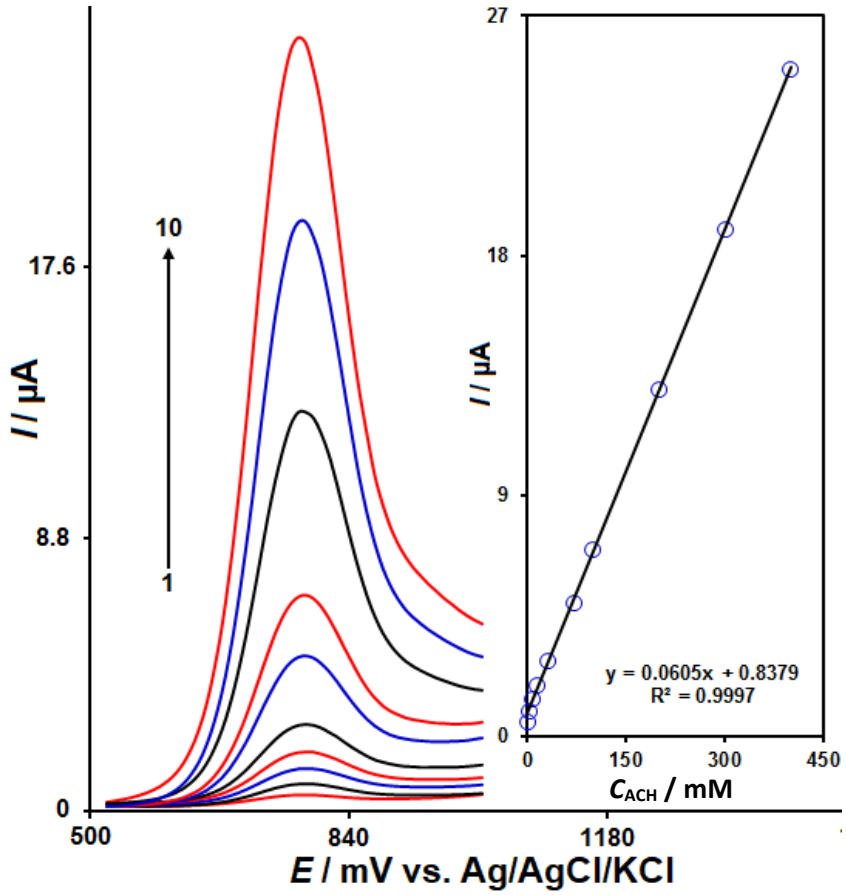

Figure 5. *The DPVs of the WO_3 NR/GCE in PBS (0.1 M, pH 7.0) containing different concentrations of ACH (1) 0.1, (2) 2.5, (3) 7.5, (4) 15.0, (5) 30.0, (6) 70.0, (7) 100.0, (8) 200.0, (9) 300.0 and (10) 400.0 µM. Inset: the plot of the current as a function of the ACH concentration in the range of 0.1 to 400.0 µM*

Real sample analysis

Urine samples were collected. The urine samples were then centrifuged at 2000 rpm for 15 minutes using 10 mL of the sample. The supernatant was filtered through a 0.45 µm filter. A certain volume of the filtered supernatant was then transferred to 25 mL volumetric flasks and diluted with 0.1 M PBS of pH 7.0 up to the mark. Different concentrations of ACH were added to spike the diluted urine and ACH tablet samples. The proposed electrochemical method was then used to analyze the ACH contents in the spiked urine samples and ACH tablets using the standard addition method. The results of this analysis are presented in Table 1.

Table 1. *Determination of ACH on the WO_3 NRs/GCE surface in real samples (*n *= 5).*

Sample	Concentration, µM		Recovery, %	RSD, %
	Spiked	Found		
ACH tablet	0	4.8	-	3.5
	1.0	5.7	98.2	2.8
	2.0	7.0	102.9	1.8
	3.0	7.7	98.7	2.2
	4.0	8.9	101.1	2.1
Urine	0	-	-	-
	5.0	5.1	102.0	2.3
	7.0	6.9	98.6	3.0
	9.0	9.1	101.1	2.1
	11.0	11.4	103.6	2.7

Conclusion

This work presents a novel electrochemical platform for the quantitative determination of ACH using a GCE modified with WO_3 NRs. The electrocatalytic performance of the WO_3 NRs-modified GCE was investigated. The obtained results demonstrated favorable sensitivity, and precision in the detection of ACH. Under optimized conditions, the DPV signal exhibited a linear relationship between the oxidation current and the concentration of ACH, ranging from 0.1 to 400.0 µM. Importantly, a low LOD of 0.025 µM was achieved. This electrochemical probe was successfully employed to monitor ACH levels in ACH tablets and urine samples. The excellent analytical performance of the WO_3 NR-modified GCE highlights its potential for practical applications in the quantitative determination of ACH in various biological samples.

References

[1] N. Chauhan, S. Chawla, C. S. Pundir, U. Jain, an electrochemical sensor for detection of neurotransmitter-acetylcholine using metal nanoparticles, 2D material and conducting polymer modified electrode, *Biosensors and Bioelectronics* **89** (2017) 377-383. https://doi.org/10.1016/j.bios.2016.06.047

[2] A. Shadlaghani, M. Farzaneh, D. Kinser, R. C. Reid, Direct electrochemical detection of glutamate, acetylcholine, choline, and adenosine using non-enzymatic electrodes, *Sensors* **19(3)** (2019) 447. https://doi.org/10.3390/s19030447

[3] S. Pitiphattharabun, K. Auewattanapun, T. L. Htet, M. M. Thu, G. Panomsuwan, R. Techapiesancharoenkij, J. Ohta, O. Jongprateep, Reduced graphene oxide/zinc oxide composite as an electrochemical sensor for acetylcholine detection, *Scientific Reports* **14(1)** (2024) 14224. https://doi.org/10.1038/s41598-024-64238-7

[4] E. Lamy, L. Pilyser, C. Paquet, E. Bouaziz-Amar, S. Grassin-Delyle, High-sensitivity quantification of acetylcholine and choline in human cerebrospinal fluid with a validated LC-MS/MS method, *Talanta* **224** (2021) 121881. https://doi.org/10.1016/j.talanta.2020.121881

[5] J. Guo, S. Wu, Y. Wang, M. Zhao, A label-free fluorescence biosensor based on a bifunctional MIL-101 (Fe) nanozyme for sensitive detection of choline and acetylcholine at nanomolar level, *Sensors and Actuators B* **312** (2020) 128021. https://doi.org/10.1016/j.snb.2020.128021

[6] J. Říčný, S. Tuček, I. Vinš, Sensitive method for HPLC determination of acetylcholine, choline and their analogues using fluorometric detection, *Journal of neuroscience methods* **41(1)** (1992) 11-17. https://doi.org/10.1016/0165-0270(92)90119-X

[7] F. J. R. Beattie, A colorimetric method for the determination of choline and acetylcholine in small amounts, *Biochemical Journal* **30(9)** (1936) 1554. https://doi.org/10.1042%2Fbj0301554

[8] S. Pitiphattharabun, K. Auewattanapun, T. L. Htet, M. M. Thu, G. Panomsuwan, R. Techapiesancharoenkij, J. Ohta, O. Jongprateep, Reduced graphene oxide/zinc oxide composite as an electrochemical sensor for acetylcholine detection, *Scientific Reports* **14(1)** (2024) 14224. https://doi.org/10.1038/s41598-024-64238-7

[9] M. Y. Emran, M. A. Shenashen, A. El Sabagh, M. M. Selim, S. A. El-Safty, Enzymeless copper microspheres@ carbon sensor design for sensitive and selective acetylcholine screening in human serum, *Colloids and Surfaces B* **210** (2022) 112228. https://doi.org/10.1016/j.colsurfb.2021.112228

[10] M. Y. Emran, M. A. Shenashen, A. Elmarakbi, M. M. Selim, S. A. El-Safty, Hierarchical engineering of Mn_2O_3/carbon nanostructured electrodes for sensitive screening of acetylcholine in biological samples, *New Journal of Chemistry* **46(32)** (2022) 15557-15566. https://doi.org/10.1039/D2NJ02390C

[11] S. Tajik, H. Beitollahi, S. Shahsavari, F. G. Nejad, Simultaneous and selective electrochemical sensing of methotrexate and folic acid in biological fluids and pharmaceutical samples using Fe3O4/ppy/Pd nanocomposite modified screen printed graphite electrode, *Chemosphere* **291** (2022) 132736. https://doi.org/10.1016/j.chemosphere.2021.132736
[12] M. Manjunatha Charithra, J. G. Manjunatha, Electrochemical sensing of paracetamol using electropolymerised and sodium lauryl sulfate modified carbon nanotube paste electrode, *ChemistrySelect* **5(30)** 2020) 9323-9329. https://doi.org/10.1002/slct.202002626
[13] S. Borji, H. Beitollahi, F.G. Nejad, Evaluating the Electrochemical Detection of Methyldopa in the Presence of Hydrochlorothiazide using a modified Carbon Paste Electrode and Voltammetric Analysis, *Topics in Catalysis* **67(9)** (2024) 773-784. https://doi.org/10.1007/s11244-023-01855-y
[14] J. G. Manjunatha, Highly sensitive polymer based sensor for determination of the drug mitoxantrone, *Journal of Surface Science and Technology* **34** (2018) 74-80. https://doi.org/10.18311/jsst/2018/15838
[15] J. G. Manjunatha, Fabrication of efficient and selective modified graphene paste sensor for the determination of catechol and hydroquinone, *Surfaces* **3(3)** (2020) 473-83. https://doi.org/10.3390/surfaces3030034
[16] S. Tajik, P. Shams, H. Beitollahi, F. Garkani Nejad, Electrochemical Nanosensor for the Simultaneous Determination of Anticancer Drugs Epirubicin and Topotecan Using UiO-66-NH_2/GO Nanocomposite Modified Electrode, *Biosensors* **14(5)** (2024) 229. https://doi.org/10.3390/bios14050229
[17] G .V. Prasad, V. Vinothkumar, S. J. Jang, T. H. Kim, Multi-walled carbon nanotube/graphene oxide/poly (threonine) composite electrode for boosting electrochemical detection of paracetamol in biological samples, *Microchemical Journal* **184** (2023) 108205. https://doi.org/10.1016/j.microc.2022.108205
[18] G. Tigari, J. G. Manjunatha. Poly (glutamine) film-coated carbon nanotube paste electrode for the determination of curcumin with vanillin: an electroanalytical approach, *Monatshefte für Chemie-Chemical Monthly* **151** (2020) 1681-1688. https://doi.org/10.1007/s00706-020-02700-8
[19] J. G. Manjunatha, A promising enhanced polymer modified voltammetric sensor for the quantification of catechol and phloroglucinol, *Analytical and Bioanalytical Electrochemistry* **12(7)** (2020) 893-903. www.abechem.com/article_43499_14e9b1642faa3cfb0d7867d6452c363b.pdf
[20] M. M. Charithra, J. G. Manjunatha, Electrochemical sensing of adrenaline using surface modified carbon nanotube paste electrode, *Materials Chemistry and Physics* **262** (2021) 124293. https://doi.org/10.1016/j.matchemphys.2021.124293
[21] K. Ladi, A. S. Ghrera, Development of electrode by using gold-platinum alloy nanoparticles for electrochemical detection of serum amyloid A protein, *Journal of Electrochemical Science and Engineering* **14(3)** (2024) 339-352. https://doi.org/https://doi.org/10.5599/jese.2036
[22] P. A. Pushpanjali, J. G. Manjunatha, B. M. Amrutha, N. Hareesha, Development of carbon nanotube-based polymer-modified electrochemical sensor for the voltammetric study of Curcumin, *Materials Research Innovations* **25** (2021) 412-420. https://doi.org/10.1080/14328917.2020.1842589
[23] Z. Sarbandian, H. Beitollahi, an electrochemical sensor based on a modified glassy carbon electrode for detection of epinephrine in the presence of theophylline, *ADMET and DMPK* **12(2)** (2024) 391-402. https://doi.org/10.5599/admet.2082
[24] H. Beitollahi, S. Tajik, S. Z. Mohammadi, M. Baghayeri, Voltammetric determination of hydroxylamine in water samples using a 1-benzyl-4-ferrocenyl-1H-[1, 2, 3]-triazole/carbon nanotube-modified glassy carbon electrode, *Ionics* **20** (2014) 571-579. https://doi.org/10.1007/s11581-013-1004-0

[25] J. G. Manjunatha, C. Raril, N. Hareesha, M. M. Charithra, P. A. Pushpanjali, G. Tigari, D. K. Ravishankar, S. C. Mallappaji, J. Gowda, Electrochemical fabrication of poly (niacin) modified graphite paste electrode and its application for the detection of riboflavin, *The Open Chemical Engineering Journal* **14** (2020) 90-98. https://doi.org/10.2174/1874123102014010090
[26] N. Hareesha, J.G. Manjunatha, Z.A. Alothman, Sillanpää M. Simple and affordable graphene nano-platelets and carbon nanocomposite surface decorated with cetrimonium bromide as a highly responsive electrochemical sensor for rutin detection, *Journal of Electroanalytical Chemistry* **917** (2022) 116388. https://doi.org/10.1016/j.jelechem.2022.116388
[27] Z. Hu, Y. Yang, X.F. Zhang, C. Xu, J. Yao, Integrating two-dimensional MXene fillers into nanocellulose for the fabrication of CO_2 separation membranes., *Separation and Purification Technology* **326** (2023) 124704. https://doi.org/10.1016/j.seppur.2023.124704
[28] J. Guo, H. Zeng, Y. Chen, Emerging nano drug delivery systems targeting cancer-associated fibroblasts for improved antitumor effect and tumor drug penetration, *Molecular Pharmaceutics* **17(4)** (2020) 1028-1048. https://doi.org/10.1021/acs.molpharmaceut.0c00014
[29] Y. Wei, Q. Wang, Q. Liu, R. Wang, Y. Wang, S. Luo, Y. Zhang, P. Hou, S. Yan, X. Liu, J. Guo, Nano hollow carbon regulated by carbon dots as a high-performance anode for potassium and sodium ion battery, *Applied Surface Science* **648** (2024) 159017. https://doi.org/10.1016/j.apsusc.2023.159017
[30] C. Li, J. Wang, Y. Yan, P. Huo, X. Wang, MOF-derived NiZnCo-P nano-array for asymmetric supercapacitor, *Chemical Engineering Journal* **446** (2022) 137108. https://doi.org/10.1016/j.cej.2022.137108
[31] T. V. H. Luu, N. T. M. Tho, T. T. T. Thuy, L. N. Thong, N. T. Dung, P. H. Dang, Synthetization pill-like C-doped ZnO nano-photocatalyst for removing ofloxacin and methylene blue under visible light, *Journal of Sol-Gel Science and Technology* **110(1)** (2024) 204-220. https://doi.org/10.1007/s10971-024-06348-2
[32] A. Sheikh, M. A. Abourehab, A. S. Tulbah, P. Kesharwani, Aptamer-grafted, cell membrane-coated dendrimer loaded with doxorubicin as a targeted nanosystem against epithelial cellular adhesion molecule (EpCAM) for triple negative breast cancer therapy, *Journal of Drug Delivery Science and Technology* **86** (2023) 104745. https://doi.org/10.1016/j.jddst.2023.104745
[33] D. Ozturk, Fe_3O_4/Mn_3O_4/ZnO-rGO hybrid quaternary nano-catalyst for effective treatment of tannery wastewater with the heterogeneous electro-Fenton process: Process optimization, *Science of the Total Environment* **828** (2022) 154473. https://doi.org/10.1016/j.scitotenv.2022.154473
[34] A. G. Oliveira, J. de Lara Andrade, M. C. Montanha, C. Y. L. Ogawa, T. K. F. de Souza Freitas, J. C. G. Moraes, F. Sato, S. M. Lima, L. H. da Cunha Andrade, A. A. W. Hechenleitner, E. A. G. Pineda, Wastewater treatment using Mg-doped ZnO nano-semiconductors: A study of their potential use in environmental remediation, *Journal of Photochemistry and Photobiology A* **407** (2021) 113078. https://doi.org/10.1016/j.jphotochem.2020.113078
[35] H. Cheng, L. Chen, D.J. McClements, J. Xu, H., Long, J. Zhao, Z. Xu, M. Meng, Z. Jin, Recent advances in the application of nanotechnology to create antioxidant active food packaging materials, *Critical Reviews in Food Science and Nutrition* **64(10)** (2024) 2890-2905. https://doi.org/10.1080/10408398.2022.2128035
[36] E. Han, Y. Pan, L. Li, J. Cai, Bisphenol A detection based on nano gold-doped molecular imprinting electrochemical sensor with enhanced sensitivity, *Food Chemistry* **426** (2023) 136608. https://doi.org/10.1016/j.foodchem.2023.136608
[37] Y. Li, Y. Yang, Y. Huang, J. Li, P. Zhao, J. Fei, Y. Xie, An ultrasensitive dietary caffeic acid electrochemical sensor based on Pd-Ru bimetal catalyst doped nano sponge-like carbon, *Food Chemistry* **425** (2023) 136484. https://doi.org/10.1016/j.foodchem.2023.136484

[38] Y. Zhang, N. Li, Y. Xu, M. Yang, X. Luo, C. Hou, D. Huo, An ultra-sensitive electrochemical aptasensor based on Co-MOF/ZIF-8 nano-thin-film by the in-situ electrochemical synthesis for simultaneous detection of multiple biomarkers of breast cancer, *Microchemical Journal* **187** (2023) 108316. https://doi.org/10.1016/j.microc.2022.108316

[39] K. Kamalasekaran, V. Magesh, R. Atchudan, S. Arya, A. K. Sundramoorthy, Development of electrochemical sensor using iron (III) phthalocyanine/gold nanoparticle/graphene hybrid film for highly selective determination of nicotine in human salivary samples, *Biosensors* **13(9)** (2023) 839. https://doi.org/10.3390/bios13090839

[40] H. Maseed, V. M. Reddy Yenugu, S. S. Devarakonda, S. Petnikota, M. Gajulapalli, V. V. Srikanth, Peroxidase-like Fe_3O_4 nanoparticle/few-layered graphene composite for electrochemical detection of dopamine, ascorbic acid, and uric acid, *ACS Applied Nano Materials* **6(19)** (2023) 18531-18538. https://pubs.acs.org/page/copyright/permissions.html

[41] C. Raril, J. G. Manjunatha, D. K. Ravishankar, S. Fattepur, G. Siddaraju, L. Nanjundaswamy, Validated electrochemical method for simultaneous resolution of tyrosine, uric acid, and ascorbic acid at polymer modified nano-composite paste electrode, *Surface Engineering and Applied Electrochemistry* **56** (2020) 415-426. https://doi.org/10.3103/S1068375520040134

[42] H. Beitollahi, M. Shahsavari, I. Sheikhshoaie, S. Tajik, P.M. Jahani, S.Z. Mohammadi, A.A. Afshar, amplified electrochemical sensor employing screen-printed electrode modified with Ni-ZIF-67 nanocomposite for high sensitive analysis of Sudan I in present bisphenol A, *Food and Chemical Toxicology* **161** (2022) 112824. https://doi.org/10.1016/j.fct.2022.112824

[43] S. Pitakrut, P. Sanchayanukun, S. Muncharoen, Determination of salicylic acid content in pharmaceuticals using chitosan@ Fe_3O_4/CPE electrode detected by SWV technique, *ADMET and DMPK* **11(2)** (2023) 175-184. https://doi.org/10.5599/admet.1682

[44] M. Shahsavari, S. Tajik, I. Sheikhshoaie, F.Garkani Nejad, H. Beitollahi, Synthesis of Fe_3O_4@ copper (II) imidazolate nanoparticles: Catalytic activity of modified graphite screen printed electrode for the determination of levodopa in presence of melatonin, *Microchemical Journal* **170** (2021) 106637. https://doi.org/10.1016/j.microc.2021.106637

[45] S. Kalia, R. Kumar, R. Sharma, S. Kumar, D. Singh, R. K. Singh, Two-dimensional layered rGO-MoS2 heterostructures decorated with Fe_3O_4 nanoparticles as an electrochemical sensor for detection of para-nitrophenol, *Journal of Physics and Chemistry of Solids* **184** (2024) 111719. https://doi.org/10.1016/j.jpcs.2023.111719

[46] S. Pareek, U. Jain, M. Bharadwaj, K. Saxena, S. Roy, N. Chauhan, an ultrasensitive electrochemical DNA biosensor for monitoring Human papillomavirus-16 (HPV-16) using graphene oxide/Ag/Au nano-biohybrids, *Analytical Biochemistry* **663** (2023) 115015. https://doi.org/10.1016/j.ab.2022.115015

[47] T. Jindal, P. Phogat, P., Shreya, S. Singh, R. Jha, Electrochemical and optical comparison of Cr^{3+}, Co^{2+}, Ag^{1+}, Hg^{1+} and Pb^{4+} doped WO_3 as a thin layer working electrode for electrochemical sensing, *Applied Physics A* **130(7)** (2024) 512. https://doi.org/10.1007/s00339-024-07666-6

[48] A. Mohammad, A. H. Asseri, M. I. Khan, T. Yoon, Flexible Electrochemical Sensor for Hydrogen Peroxide Detection by Employing WO_3/g-C_3N_4 Nanostructures, *Journal of The Electrochemical Society* **170(5)** (2023) 057506. https://doi.org/10.1149/1945-7111/acd1bf

[49] S. Manjunatha, N. S. Pavithra, M. Shivanna, G. Nagaraju, C. R. Ravikumar, Synthesis of Citrus Limon mediated SnO_2-WO_3 nanocomposite: Applications to photocatalytic activity and electrochemical sensor, *Journal of Environmental Chemical Engineering* **8(6)** (2020) 104500. https://doi.org/10.1016/j.jece.2020.104500

[50] G. M. Hingangavkar, Y. H. Navale, T. M. Nimbalkar, R. N. Mulik, V. B. Patil, Hydrothermally engineered WO_3 nanoflowers: A selective detection towards toxic NO_2 gas, *Sensors and Actuators B: Chemical* **371** (2022) 132584. https://doi.org/10.1016/j.snb.2022.132584

[51] S. Tajik, P. Mohammadzadeh Jahani, Voltammetric quantification of D-penicillamine by using a Carbon Paste Electrode modified with WO_3 nanorods and ionic liquid as an efficient Electrochemical sensing platform, Topics in Catalysis **67(9)** (2024) 853-863. https://doi.org/10.1007/s11244-023-01874-9